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(19) (CA) APPLICATION FOR CANADIAN PATENT (12)

(54) Recombinant Adenovirus and Process for Producing the Same

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BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a recombinant adenovirus bearing a hybrid promoter (CAG promoter) comprising a cytomegalovirus enhancer, a chicken β-actin promoter, a rabbit β-globin splicing acceptor and a polyA sequence, and a DNA sequence encoding a desired polypeptide. The present invention also relates to a process for producing the recombinant adenovirus and use thereof, particularly, use thereof in gene therapy.

Related Art Statement

Researches on adenovirus have been focused intensively on its use as a vector in experiments for transducing foreign genes, since an adenoviral vector shows an transduction efficiency of almost 100% in a variety of animal cells. In addition, the adenoviral vector has been also considered to be highly effective as a vector, because the introduced foreign gene can be examined for its functions under the condition that the transduced cells are not killed, and because animal species available as a host cell are extended over a wide range including mouse and rat.

The adenovirus, however, was really valued for its use as a vector, only after a genetic treatment

applicable over an extremely wide range of host cells, suggesting that a gene therapy involving an adenovirus would be established as a major technique in the near future.

Adenovirus is originally known to cause cold conditions in human. Therefore, there is a concern that administration of adenovirus in a large dose might invite inflammation. In fact, at the initial clinical stage, the attempt of a gene therapy using an adenovirus by Crystal's group was said to be temporarily interrupted by the side effect of pneumonia in a patient who was administered to the lung in a large dose of adenovirus. Observation was made also in experiments using cultured cells that viral infection in a high dose caused side effects attributable directly to virions, e.g., cells become round and detached in the case of adherent cells. These observations cast a significant doubt that side effects accompanied by administration of viral solution in a high dose might be caused in a practical therapy. It is therefore necessarily required to provide a combination with a promoter exhibiting a high expression level so as to ensure an expression at a sufficient level using a viral solution in a low dose. This approach is considered a key to solve the foregoing problems in applying adenovirus to a gene therapy.

In recent years, with advanced genetic recombination technique, enormous progress has been seen in the production of valuable substances utilizing such

gene transduction can infect cells ubiquitously in the resting phase, and thus considered that an adenovirus having a potent promoter integrated in the genome thereof would enable the expression of a gene in animal cells over a wide range. Therefore, recombinant adenoviruses bearing a variety of potent promoters have been prepared and examined for a gene expression in various host cells. As a result, it has been succeeded to obtain an adenovirus showing a potent promoter activity to achieve a gene expression in almost all cells. Based on the finding, further investigations have been made to accomplish the present invention.

An object of the present invention is to provide a recombinant adenovirus having a promoter exhibiting a potent activity over a wide range of animal cells, particularly a recombinant adenovirus further having a nucleotide sequence encoding a desired foreign polypeptide, more particularly, a recombinant adenovirus for use in gene therapy in which a human defective gene has been inserted.

Another object of the present invention is to provide a process for producing such a recombinant adenovirus in a simple manner.

That is, the present invention is characterized by the following features:

(1) A recombinant adenovirus bearing in the genome thereof a nucleotide sequence encoding a desired foreign polypeptide and a hybrid promoter (CAG

(8) A recombinant adenovirus bearing in the genome thereof a nucleotide sequence encoding a desired foreign polypeptide and a foreign promoter which are inserted into the genome in an orientation to the left hand.

(9) A recombinant adenovirus according to the above (8), wherein said adenovirus genome is deleted of at least 1.3 to 9.3% segment including E1A gene region.

(10) A recombinant adenovirus according to the above (9), wherein said nucleotide sequence encoding a desired foreign polypeptide and said promoter are inserted into the E1A-deleted gene region.

(11) A recombinant adenovirus according to the above (10), wherein said adenovirus genome is deleted of at least 79.6 to 84.8% segment including E3 gene region.

(12) A process for producing a recombinant adenovirus having an expression unit inserted in the genome which comprises the steps of:

mixing a cosmid constructed by deleting at least 1.3 to 9.3% segment including E1A gene region in the adenovirus genome and inserting the expression unit into the E1A-deleted gene region, with an adenovirus DNA-terminal protein complex digested with a restriction enzyme at 3 to 10 sites in the left end of the adenovirus genome; and

transfecting with the resulting mixture a cell lin which expresses the E1A gene.

DETAILED DESCRIPTION OF THE INVENTION

An adenovirus used in the present invention is a human adenovirus which utilizes a human body as a natural host. The adenoviral genome is a double stranded DNA of about 36 kbp and takes a peculiar structure in that both ends of the DNA strand have an inverted repeat sequence of about 100 bp and that 55 kDa protein which is processed from E2B gene product is covalently bound to the 5' end of each of both ends of the DNA strand. This peculiar structure of the genome has been a serious drawback for a vector as described below. The present invention is characterized by providing a novel and useful recombinant adenovirus and a process for production thereof which have overcome the serious drawback.

The recombinant adenovirus of the present invention is a kind of recombinant vectors which are suitable for transfection in an adenovirus-infectious eukaryotic system, particularly in a human or animal cell system. The recombinant adenovirus of the present invention is characterized in that it is deleted of E1 gene region, particularly E1A gene region involving the induction of neoplasm and hence cannot propagate in host cells, except for a cell line constitutively expressing the E1A gene such as a human fetal kidney-derived cell line (293 cell).

When inoculated on the 293 cells, the recombinant adenovirus particles of the present

adenovirus can not propagate, as far as the virus particles can infect and be internalized into cells.

The genome in the recombinant adenovirus of the present invention is also advantageous as a vector in that, even though the genome is not replicated extrachromosomally, the genome is retained in the nucleus over two weeks to two months to express the foreign gene considerably persistently.

The genome in the recombinant adenovirus of the present invention is characterized by having a hybrid promoter (CAG promoter) inserted therein comprising a cytomegalovirus enhancer, a chicken β-actin promoter, a rabbit β-globin splicing acceptor and a polyA sequence derived from a rabbit β-globin.

This hybrid promoter (CAG promoter) is disclosed as a high level expression vector in Japanese Patent KOKAI (Laid-Open) No. 3-168087. The hybrid promoter can be prepared by excising from pCAGGS described in Japanese Patent KOKAI supra at page 13, line 20 to page 20, line 14 and page 22, first line to page 35, line 6, with restriction enzymes SalI and HindIII. The hybrid promoter thus produced can be used in the present invention. The present inventors made comparative study on the expression level of lacZ gene with conventional several promoters and found out that the hybrid promoter (CAG promoter) in the recombinant adenovirus is more excellent in the expression level (as shown in Comparative Example hereinafter). Based on th

host cells to secrete the thus expressed polypeptide into the culture medium.

The genome in the recombinant adenovirus of the present invention is deleted of the E1 gene region, especially the E1A gene region. Thus, the recombinant adenovirus is defective in the E1A gene region which is associated with the neoplastic transformation activity of adenovirus, whereby the recombinant adenovirus according to the present invention is rendered avirulent and only the foreign nucleotide sequence inserted therein is selectively expressed. The entire E1 gene region is not necessarily deleted, but the deletion of the 1.3 to 9.3% segment including the E1A gene region can exhibit the desired effects as stated above. A nucleotide sequence encoding a desired foreign polypeptide and a foreign promoter in the recombinant adenovirus of the present invention are preferably inserted into the E1A-deleted gene region.

Further, the E3 gene region can be also deleted from the genome in the recombinant adenovirus of the present invention. In particular, the deletion of 79.6 to 84.8% segment including the E3 gene region is preferable. This segment is not required for replication of the foreign nucleotide sequence.

A chicken β-actin promoter which is used to be inserted into the genome of the recombinant adenovirus of the present invention originally exhibits a potent promoter activity without any modifications. The

present invention is extremely useful for gene therapy.

Examples of animal cells to be infected with the recombinant adenovirus ... human or mammal lung epithelial cells, gastrointestinal epithelial cells, neuronal cells, liver and muscles (skeletal muscle, heart muscle).

Hereunder, the process for producing the recombinant adenovirus of the present invention is explained in detail.

In general, it is extremely difficult to produce the recombinant adenovirus of the present invention, because proteins are covalently linked to the both termini of the adenovirus genome as described hereinabove.

Therefore, the following procedures are preferably used in the present invention.

(1) Firstly, from the entire length of adenoviral genome (36 kb), prepared is a cosmid bearing about 31 kb genomic DNA deleted of the E3 gene region (1.9 kb) and the E1A·E1B gene region (2.9 kb), each of which is not required for replication in 293 cells. To the E1A·E1B-deleted gene region in the cosmid, an expression unit is inserted which contains a foreign promoter, a nucleotide sequence encoding the desired foreign polypeptide to be expressed and a polyA sequence. The expression unit is preferably inserted in an orientation to the left hand.

The cosmid is prepared using lambda in vitro

restriction enzymes including NsiI and AvaIII which have the same recognition site as that of EcoT22I. The thus obtained genome is used as a parent viral genome.

(3) Then the cosmid bearing the desired expression unit as stated above is mixed with the parent viral DNA-TPC followed by transfection to 293 cells (ATCC No. CRL-1573) according to a conventional calcium phosphate co-precipitation method.

In the 293 cells, firstly, homologous recombination occurs in the homologous sequences between the two molecules, i.e., the parent viral DNA and the cosmid. Then, the molecule which has initiated replication from the right end (opposite to the E1 gene region) repairs the left end sequence by cutting off the plasmid sequence utilizing the intact right end sequence. As described above, this is because the adenovirus genome has 102 base pair inverted terminal sequences at both ends thereof, both of which are identical. This mechanism suggests that even if the one end of the adenovirus genome is attacked by an exonuclease, the adenovirus genome by itself would repair using the sequence at the other end.

(4) After the foregoing procedures have been conducted, a desired recombinant adenovirus may be collected from the adenovirus propagated in the 293 cells. According to the process of the present invention, any selection markers are not required, because of a highly efficiency of generating the desired

cells to obtain the virus propagated therein, from which DNA is extracted followed by digestion with restriction enzyme XhoI and examination of the resulting digestion patterns. Where the solution is suspected to be contaminated with the deleted virus or the parent virus, the solution is discarded.

In order to make the characteristic features of the present invention clearer, the process of the present invention is compared below with the conventional process according to Melissa A. Rosenfeld et al., Cell vol. 68, 143-155 (1992).

According to the process of the present invention, the cosmid bearing almost the entire length of adenovirus DNA is employed, whereas in the conventional process a cassette of about 5 kb near the E1A·E1B gene region was used. By using such a specific cosmid cassette, the frequency of homologous recombination can be markedly improved in the process of the present invention.

According to the process of the present invention, the terminal proteins-bound genomic DNA (DNA-TPC) is employed as the parent viral DNA, whereas in the conventional process the terminal proteins-deleted DNA was used. This difference results in that the process of the present invention is several tens times more excellent in the efficiency than the conventional process.

Reference Examples and Comparative Examples but is not deemed to be limited thereto.

In the Examples, various operations for handling phage, plasmid, DNA, various enzymes, E. coli, culture cells and the like were carried out according to the methods as described in Molecular Cloning, A Laboratory Manual., edited by T. Maniatis et al., second edition (1989), Cold Spring Harbor Laboratory, unless otherwise indicated.

Example 1

Construction of recombinant adenovirus

The recombinant adenovirus is constructed roughly by three steps. That is, the construction comprises the steps of inserting the expression unit into the cosmid, producing the parent virus DNA-TPC and co-transfecting the 293 cells with the cosmid and the DNA-TPC followed by isolation and purification. Each of the steps is explained below in more detail.

(1) Insertion of the expression unit into the cosmid

(i) Firstly, 0.2 µg of an expression unit fragment with blunt ends was mixed with 1 µg of pAdex1w DNA previously digested with SwaI.

In this Example, a set of LacZ gene and the hybrid promoter (CAG promoter) comprising cytomegalovirus enhancer, chicken β-actin promoter, rabbit β-globin splicing acceptor and polyA sequence was used as the expression unit. For making the blunt end,

reaction. Sterilized water and a buffer solution for SwaI reaction were added thereto to make the whole volume 48 µl. Then ligase was inactivated with heating at 70°C for 10 minutes.

Unlike a plasmid, macromolecules of the tandemly-ligated cosmid are much more efficiently packaged in a phage particle than the circularly-ligated molecules.

(iv) After adding 2 µl of SwaI enzyme (Boehringer), digestion was carried out at 25°C for an hour.

If the cosmid is re-ligated without the insert of the expression unit therein, the SwaI recognition site will be regenerated. The digestion with SwaI is performed to re-cleave the cosmid having no expression unit inserted therein, so that no colony is formed. This is a potential method for selecting only the cosmid having the insert.

(v) The cosmid was subjected to phenol extraction, centrifugation and gel filtration according to a conventional method as described in Molecular Cloning, vol. 3, E.34.

(vi) Again digestion was performed with SwaI. That is, 5 µl of SwaI enzyme was added to the buffer for the SwaI reaction followed by the cleavage of the cosmid at 25°C for 2 hours. The cleavage was conducted because of the reason as explained above.

(ix) The orientation and structure of the expression unit inserted were confirmed by digestion with restriction enzymes.

Using NruI and ligase, a plasmid bearing the expression unit but deleted of most adenovirus DNA was prepared, and DNA was then prepared from the plasmid for final confirmation of cDNA cloning. At the same time, the expression of the desired gene, LacZ gene, was confirmed by transient expression in COS cells. As a matter of course, this plasmid was not used but the cosmid was used for producing the recombinant adenovirus.

(2) Production of adenoviral DNA-protein complex (Ad5 dlX DNA-TPC)

(i) As adenovirus DNA, Ad5 dlX (I. Saito et al., J. Virology, vol. 54, 711-719 (1985)) was used. Ad5 dlX was infected to HeLa cells (at the amount of 10 Roux bottles) followed by incubation.

That is, the viral stock of Ad5-dlX (up to 10^9 PFU/ml) was infected in 0.2 ml/Roux. Three days after, the detached floating cells were collected by centrifugation at 1500 rpm for 5 minutes. Most of the adenovirus particles were present in the nucleus, but not in the medium. The virus is therefore advantageously purified from the infected cells.

The following procedures were aseptically performed.

(v) The band of virus was fractionated and mixed with an equimolar amount of 8M guanidine hydrochloride. Furthermore, 4M guanidine hydrochloride-saturated cesium chloride was added to the mixture. The resulting mixture was filled in a VTi65 tube. The particle protein was denatured by 4M guanidine hydrochloride to cause dissociation, whereby the DNA-TPC was released. Ethidium bromide could not be used in this experiment, because any procedure for removing the ethidium bromide used has not been established.

(vi) The tube described above was subjected to ultracentrifugation at 15°C overnight at 55 krpm, followed by fractionation with 0.2 ml. From each of the fractions, 1 µl was mixed and then fluorescence-stained with 1 µg/ml of ethidium bromide aqueous solution to confirm the presence or absence of DNA. Two to three fractions containing DNA were collected.

(vii) The fractions were twice dialyzed against 500 ml of TE overnight. The fraction tubes were then stored at -80°C. The amount of the thus obtained Ad5dlX DNA-TPC was determined by OD260 in the same way as in conventional DNA.

(viii) The resulting Ad5dlX DNA-TPC was digested with a sufficient amount of EcoT22I for 2 hours and then stored at -80°C for the construction of recombinant adenovirus at the following third steps.

The DNA-TPC could undergo digestion with restriction enzyme, dialysis and gel filtration, but

using CellPhect transfection Kit (Pharmacia) according to the calcium phosphate method. The mixture was dropped onto the medium in the 6 cm culture dish to continue the incubation.

After the overnight incubation (for about 16 hours), the culture medium was changed in the next morning. Then, in the evening, the medium was poured at 0.1 ml/well with 5% FCS-containing DMA into wells in three 96-well collagen coated plates (stock, 10-fold dilution, 100-fold dilution). In order to avoid a significant difference in the cell number between each plate, a mixture with the 293 cells on the 10 cm culture dish was simultaneously inoculated on plate.

(iii) Three to four days after and eight to ten days after, 50 µl of 10% FCS-containing DME was further added to each well. When the 293 cells became thin, 10% FCS-containing DME was added to the well earlier.

The wells in which the virus propagated and the cells were dead appeared in 7 to 15 days. Every time when the cells in the well were completely dead, each of the culture media was aseptically transferred to a sterilized 1.5 ml tube with a sterile posteur pipette. The tube was quickly frozen and stored at -80°C.

(iv) The judgment was completed in 15 to 18 days. About ten (10) tubes were selected from the tubes in which the cells were dead at a relatively late stage. After six (6) cycles of the freeze-thawing,

precipitation gave nucleic acid. The nucleic acid was dissolved in 50 µl of TE containing 20 µg RNase/ml.

After 15 µl of the solution was digested with XhoI containing CG in the recognition site for digesting the expression unit, the digested product was subjected to electrophoresis overnight on agarose gel having a length of about 15 cm. The patterns thus obtained were compared. Selected were the bands exhibiting accurately the sequence from the digested site in the expression unit to the left end (E1 gene region side) of the adenovirus genome. The clones which exhibited an unexpected band were discarded, since there was a possibility that the clones would be contaminated with the virus having deletions.

The adenovirus DNA propagated up to at a level of 10,000 copies/cell. Accordingly, the entire DNA could be extracted together with cell DNA and digested with restriction enzymes to observe the bands of viral DNA. The restriction enzyme such as XhoI containing CG in the recognition site does not digest the cellular DNA and as the result, the pattern could be readily observable. In the case of other enzymes, the non-infected 293 cellular DNA was required for control. The bands derived from human repetitive DNA were observed.

(viii) The second seed stock of the desired virus strain identified by the XhoI digestion, was infected in an amount of 0.1 ml to the 293 cells charged

ly, the desired virus was purified from the first seed solution by a limiting dilution method.

Reference Example 1

Simple assay for the titer of the recombinant adenovirus of the present invention

The titer of the recombinant adenovirus according to the present invention may be assayed in a simple manner according to the following procedures.

(1) One 10 cm culture dish charged with the 293 cells is prepared.

The recombinant adenovirus solution (i.e., the third seed solution) is serially diluted to 10^{-1} to 10^{-4} using 5% FCS-supplemented DME, for example, 0.9 ml of DME + 0.1 ml of the virus solution. The micropipette tips are all exchanged.

(2) In all wells of one collagen-coated 96-well plate, 5% FCS-supplemented DME is charged by 50 µl each.

On the first row, 25 µl each of the recombinant adenovirus diluted to 10^{-4} is charged.

Using a eight (8)-channel pipette, 25 µl is transferred to the wells on the second row. Thereafter the same operation is repeated until the 11th row and the last 25 µl is discarded. As the result, the 3rd serial dilutions may be prepared until $3^{11} \times 10^{-4}$. The 12th row is non-infected cells for control.

Micropipette tips used in this case are

are shown in Table 1. In this case, the titer was assayed as follows:

The volume of the diluted virus solution was 50 µl. When the titer was 1 PFU in 50 µl at this concentration, the titer of the viral stock was:

$$1\ ml \div 50\ \mu l = 10^{-7.817} = 20 \times 10^{7.817}$$
$$= 10^{9.118}$$
$$= 1.3 \times 10^{9}\ (PFU/ml)$$

That is, when a half of the 8th row showed cytopathic effects, the titer was 1.3×10^9 PFU/ml and when cytopathic effects occurred up to a half of the 7th row, the titer reached 1/3 of the above titer, that is, 4.4×10^8 PFU/ml.

Reference Example 2

Construction of pAdex1c and pAdex1w

We prepared cosmid, pAdex1c, in the following manner.

(1) Preparation of pUAF0-17D which contains left terminal 17% fragment of adenovirus genome with deletion of E1 gene region.

Adenovirus type 5 DNA treated with S1 nuclease and rendered blunt-end fragment was purified by phenol extraction and ethanol precipitation. The blunt-end fragment was ligated with BamHI linker. The ligated fragment was digested with HindIII and DNA sample was separated by electrophoresis in agarose gel. The fragment of 2.8 kb size (left terminal 8% fragment of Adenovirus genome) was recovered by electroelution from gel slices and inserted into BamHI/HindIII digested pUC19. Resulting plasmid was named pUAF0-8.

Adenovirus type 5 DNA was digested with HindIII and DNA sample was applied on the agarose gel electrophoresis. The fragment of 3.4 kb size (8-17% fragment of Adenovirus genome) was recovered and inserted into HindIII digested pUC19. Resulting plasmid was named pUAF8-17.

The PvuII site at nucleotide position 454 in pUAF0-8 was converted to a ClaI site by using ClaI linker. Then, the DNA sample was digested with Bam HI/ClaI and the resulting Bam HI-ClaI fragment (454 nucleotide(nt)) was purified through the agarose gel electrophoresis.

Sci., vol. 83, p8664-8668, 1986), charomid9-11 was digested with Asp718 and BamHI, filled in by Klenow fragment of DNA polymerase I. and self-ligated.

After transformation, the resulting charomid was named charomid6-11 which lacks KpnI, SmaI and BamHI sites.

New BamHI site was regenerated by using BamHI linker at EcoRI site (EcoRI site also regenerated). The resulting charomid was named chdRBR7-11.

(5) Preparation of pAdex1c

To construct pAdex1c, the BamHI-Bst1107 fragment (2.9 kb; of pUAF0-17D, the Bst1107-EcoRI fragment (21.6 kb) of adenovirus genome, the EcoRI-SwaI fragment (6.5 kb) of pX2W and the EcoRI/Ec136I digested chdRBR7-11 were ligated. After ligation, the reaction mixture was packaged <u>in vitro</u> and adsorbed to DH5α cells. pAdex1c was selected from transformants.

(6) Preparation of pAdex1w

pAdex1w was constructed from pAdex1c by converting the ClaI site to SwaI site.

<u>Comparative Example 1</u>

<u>Influence of various promoters on expression of LacZ gene for various established cell lines</u>

Recombinant adenoviruses were prepared by substituting SRα promoter (SV40 early promoter + HTLV1J'LTR) r EF-1α promoter (derived from human polypeptide 1 ngation factor gene) for the promoter

plasmid was digested with SalI and HindIII and the resulting fragment was blut-ended.

The plasmid was ligated with pAdex1w previously digested with SwaI to prepare an expression cosmid. The adenovirus which contains the expression unit having an orientation to the left hand was selected and used for constructing the recombinant adenovirus.

(2) For the insertion of SRα promoter and LacZ gene into the adenovirus genome, pMC1871 was used as a material for LacZ gene in the same way as in the CAG promoter. After digesting with SmaI at the 5' end, NotI linker was ligated and then digested with PstI downstream of a termination codon. The ends were rendered blunt and then ligated with KpnI linker. The LacZ fragment was ligated with the synthetic DNA as described above to be cloned between the PstI site and the KpnI site of the expression vector pcDL-SRα296 bearing SRα promoter (pSRLacZ).

To prepare the recombinant adenovirus (Adex1SRLacZ) for expressing LacZ under control of SRα promoter, the expression unit of SRα-LacZ-polyA was excised from pSRLacZ using HindIII and TthlllI and rendered blunt. The expression unit was then inserted into Adex-producing cassette, Adex1W, at the SwaI site. The adenovirus which contains the expression unit having an orientation to the left hand was selected and used for constructing the recombinant adenovirus.

washed twice with PBS. Then 0.5 ml of buffer for extraction was added thereto. After ultrasonication with 30 second intervals for 90 seconds in total, 0.5 ml of 80% glycerol was added and the resulting mixture was centrifuged at 15000 rpm for 10 minutes. The thus obtained supernatant was used as the cell extract.

To 850 µl of a reaction mixture containing 1.0 ml of 0.5 M sodium phosphate buffer (pH 7.8), 1.0 ml of 0.5 M β-mercaptanol, 1.0 ml of 10 mM $MgCl_2$ and 5.5 ml of distilled water was added 50 µl of the cell extract, the cell extract buffer (for blank control) or β-galactosidase solution (for control), followed by preincubation for 5 minutes. Then, a substrate solution was further added thereto. As the substrate, o-nitrophenyl-β-galactoside was used.

After incubation for 30 minutes, 400 µl of a reaction-terminating solution was added and absorbance was measured at 420 nm. The activity was determined according to the following equation.

Where absorbance exceeds 1, it is preferred that the cell extract solution is diluted with extraction buffer and the absorbance is again measured.

Units/ml = (absorbance of sample - absorbance of blank)/4.5l[a] x 1.4 ml[b] x 1/30 min[c] x 1.0 ml/0.05 ml[d]

wherein:

a): The concentration µmole/ml of the ensyme reaction product is determined by dividing with extinction coefficient of o-nitrophenol,

Comparative Example 2

Influence of orientation on expression of LacZ and HCV gene for various promoters. Recombinant adenoviruses having different promoters and genes in opposite orientations were prepared, and compared with each other in the activity for virus production.

(1) Construction of HCV expressing recombinant adenovirus

The cDNA fragment of 2.2 kb size (nucleotide number; 307-2554) was isolated by polymerase chain reaction and was inserted between the PstI and the KpnI site of the pcDL-SRα296 in the same manner of LacZ cDNA.

Recombinant adenovirus was also prepared in a similar way.

(2) Construction of LacZ expressing recombinant adenovirus

The preparation was as same as Comparative Example 1.

The results obtained are shown in Table 2. The results reveal that the leftward in the present invention exhibits a much more potent production activity in three cases, than in rightward.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A recombinant adenovirus bearing in the genome thereof a nucleotide sequence encoding a desired foreign polypeptide and a hybrid promoter (CAG promoter) comprising a cytomegalovirus enhancer, a chicken β-actin promoter, a rabbit β-globin splicing acceptor and a polyA sequence.

2. A recombinant adenovirus according to claim 1, wherein said adenovirus genome is deleted of at least 1.3 to 9.3% segment including E1A gene region.

3. A recombinant adenovirus according to claim 2, wherein said nucleotide sequence encoding a desired foreign polypeptide and said CAG promoter are inserted into the E1A-deleted gene region.

4. A recombinant adenovirus according claim 3, wherein said adenovirus genome is deleted of at least 79.6 to 84.8% segment including E3 gene region.

5. A recombinant adenovirus according to any one of claims 1 through 4, wherein said nucleotide sequence encoding a desired foreign polypeptide and said CAG promoter are inserted into the genome in an orientation to the left hand.

6. A recombinant adenovirus according to any one of claims 1 through 5, wherein said nucleotide sequence encodes a desired foreign polypeptide which has a property that, when the polypeptide is expressed under control of CAG promoter in a natural host cell, the expressed polypeptide is secreted in the culture medium.

13. A process according to claim 12, wherein said cell line is a human fetal kidney-derived cell line.

14. A process according to claim 12, wherein said expression unit comprises a nucleotide sequence encoding a desired foreign polypeptide and CAG promoter comprising a cytomegalovirus enhancer, a chicken β-actin promoter, a rabbit β-globin splicing acceptor and a polyA sequence.

15. A process according to claim 12, wherein said restriction enzyme is EcoT22I or, NsiI or AvaIII having the same recognition site as that of EcoT22I.

16. A process according to claim 12, wherein said expression unit is inserted into the genome in an orientation to the left hand.

17. A process according to claim 12, wherein each of the adenovirus genome in said cosmid and said adenovirus DNA-terminal protein complex is deleted of at least 79.6 to 84.8% segment including E3 gene region.